Development of Behavioral Tests for the Assessment of Neurologic Effects of Lead in Sheep*

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Reports of neurologic impairment of children following recovery from acute lead encephalopathy have raised questions concerning the effects of chronic low-level lead exposure on the central nervous system. Behavioral toxicologic techniques have been employed to assess the effects of lead on the central nervous system in sheep. Mature sheep receiving daily doses of 100 mg lead/kg showed a significant decrease in performance on an auditory signal detection task. Daily oral doses of 120 and 230 mg lead/sheep for 27 weeks did not alter the performance of mature sheep on a fixed-interval schedule of reinforcement behavioral task. Prenatal exposure to maternal blood lead levels of 16 or 34 μg/100 ml during gestation and postnatal daily ingestion of 16, 8, 4, or 2 mg lead/kg did not alter performance of lambs on a closed-field maze task. Slowed learning was demonstrated in lambs prenatally exposed to maternal blood lead levels of 34 μg/100 ml during gestation when tested on nonspatial, two-choice visual discrimination problems at 10–15 months of age.

A recent review (*1*) of the reported psychologic sequelae of lead ingestion in children concluded that none of the studies provided sufficient data to determine if decreased mental ability is associated with subclinical or low-level lead exposure. This question has far-reaching importance, since many children have been reported to have blood lead levels above 40 μg/100 ml (*2–4*), which by current diagnostic standards (*4*) reflects excessive lead exposure.

Behavioral toxicology is a discipline which utilizes the knowledge of animal behavior to study the selective toxicity of neuropoisons (*6*). Several studies employing behavioral toxicologic methods in the examination of the neurologic effects of lead have been reported.

Brown et al. (*7*) reported that either three or four daily doses of 111 mg lead acetate/kg given intraperitoneally did not significantly alter learning and memory in rats tested in a water T-maze. The rats ranged from 8 days to 5 weeks of age at the time of lead exposure.

Residual learning disabilities in a T-maze were demonstrated in 8- to 10-week-old rats which had nursed lead-exposed mothers for the first 3 weeks of life. The maternal rats

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received 17.5, 25.0, or 35.0 mg lead/kg daily for the first 20 days following parturition (*8*).

Doses of 15 to 20 mg tetraethyllead administered intraperitoneally did not affect the learning ability of 150-g rats on a water T-maze (*9*).

When 10- to 15-month-old rhesus monkeys were administered lead acetate at 0.05, 0.50, or 5.00 mg/kg for 30 months, Goode et al. (*10*) reported no effect on performance of (1) a conditioned response test used to evaluate the acquisition and retention of learned behavior and (2) a delayed response test which examined short-term memory and sensorimotor response. Blood lead residues of the high-exposure group ranged from 45 to 60 μg/100 ml for the last 21 weeks of the study.

Four behavioral tasks have been used in our laboratory in an attempt to find tests which are sensitive for detecting lead-induced behavioral changes. The experimental animal used in these comparative medicine studies has been the sheep.

Our use of an auditory signal detection task which required sheep to depress a pedal within 5 sec after a 0.1-sec duration of 5 kHz tone to assess the neurologic effects of lead has been reported (*11*). Adult sheep given oral lead at 100 mg/kg-day for 9 weeks performed significantly poorer on the auditory signal detection task for a food reinforcement, but this effect was associated with the anorexia of acute toxicosis. However, performance during the first 4 weeks of lead exposure, a period prior to the onset of clinical toxicosis, was significantly less stable in the exposed sheep than in the unexposed controls.

The performance of sheep on a fixed interval (FI) schedule of reinforcement during 27 weeks of lead exposure has been reported (*11*). Three groups of five adult sheep were fed sufficient lead daily to maintain blood lead levels of 6, 17, and 30 μg/100 ml for the control, low, and high lead groups, respectively. In the FI reinforcement schedule task the sheep received food reinforcement for the first pedal press after a 30-sec interval had elapsed. Pedal presses during the 30-sec interval did not result in reinforcement. After each reinforcement was given, the next 30-sec interval was started. Sheep were tested for one 15-min period per day. There was no significant difference between the number of reinforcements achieved by the three groups. A significant difference in the mean number of pedal presses between the low-lead and high-lead groups was attributed to chance.

A closed-field maze behavioral task was utilized in a two-part experiment to assess the neurologic ability of lambs following lead exposure. A modification (*12*) of the Hebb-Williams (*13*) closed-field maze was adapted for use with sheep. The modified maze apparatus was a square enclosure 16 ft on each side. The side walls and the movable panels positioned within the maze field were 4 ft high. A start box and goal box were positioned on the outside of opposite corners of the maze. The start box was a small enclosure with a door leading to the maze field. The lamb's mother was located out of view just beyond the goal area. The movable panels or barriers were positioned in the maze field to form cul-de-sacs and visual obstacles around which the lambs maneuvered to reach the goal area. The pattern of the movable barriers was different for each problem. Entrances into specific areas of the maze away from the correct solution were counted as errors.

A testing trial began with the lamb in the start box and the lamb's mother in the goal area. The lamb was released from the start box and allowed to find its way across the maze field to the goal box. The total time required to successfully traverse the maze and the total number of errors scored were recorded for each trial. At 10 days of age, the lambs were placed in the maze field for approximately 10 min for 3 consecutive days to allow acclimation to the maze apparatus. On the following 4 days, the lambs were run on several practice problems with simple visual solutions.

In the first part of the experiment, 6, 8, and 6 lambs from ewes which had re-

ceived 0 (control), 2.3 (low), or 4.5 (high) mg lead/kg-day, respectively, throughout gestation were tested in the maze (*14*). Mean blood residues in the ewes during pregnancy were 5, 18, and 34 μg/100 ml for the control, low, and high groups, respectively. Lead exposure to the dams was terminated at parturition. The blood lead level was determined once for each lamb between 2 and 4 weeks of age and again between 10 and 12 weeks of age. Mean blood lead for control lambs at 2 to 4 weeks of age was 6 μg/100 ml, while the low- and high-exposure lambs averaged 17 and 25 μg/100 ml, respectively. At 10 to 12 weeks, mean blood lead levels were 4, 9, and 14 μg/100 ml for the control, low, and high lambs, respectively. These lambs were tested on a series of 15 closed-field problems. Analysis of variance for the mean latency per trial and of the mean number of errors per trial across all 15 problems revealed no significant effect of prenatal lead exposure. These lambs were later tested on the visual discrimination task described later herein.

The second part of the experiment involving the closed-field maze examined the neurologic effects of postnatal oral lead exposure in young lambs. Five groups of four lambs each received 0, 2, 4, 8, or 16 mg lead (as the acetate)/kg starting at 5 days of age. The lambs were dosed on a 5-day/week basis for 12 weeks. Mean blood lead levels in the five groups of lambs were 15, 57, 81, 123, and 162 μg/100 ml, respectively, at 8 weeks of age. These postnatally-exposed lambs were tested on a series of 10 closed-field maze problems. Analysis of variance for the mean errors per trial revealed no significant effect of postnatal lead exposure. A significant ($P < 0.05$) treatment effect on the latency per trial existed between the control lambs and those receiving 4 mg lead/kg. No other significant differences were demonstrated. Because the significant treatment effect was not linear with dose and a biphasic response appeared unlikely, this treatment effect was attributed to chance.

The inability to demonstrate neurologic deficiencies in performance of both the prenatally and postnatally lead-exposed lambs on the closed-field maze problems can be explained by one or more of the following hypotheses: (1) the closed-field maze task was not sensitive enough to detect the neurologic impairment which existed; (2) lead exposure caused a neurologic impairment which was not evident in the lambs less than 3 months of age; or (3) the described levels of prenatal and postnatal lead exposure had no neurologic effects on the lambs.

Several of the reports of neurologic sequelae in children following lead encephalopathy utilized specialized psychologic tests to demonstrate and possibly quantitate residual brain damage (*15–17*). Visual-motor and perceptual-motor skills were frequently mentioned as areas of impairment. The study reported here was conducted to determine if changes in visual-motor skills, as measured by performance on a visual discrimination task, existed in the offspring of ewes ingesting subclinical levels of lead throughout gestation (*18*). The three groups of lambs in this experiment were the same groups as previously tested in the first closed-field maze study.

When 5 months old, four control, eight low-exposure, and six high-exposure lambs were trained to perform on a simultaneous two-choice, nonspatial visual discrimination task (*19*). Performance on six different visual discriminations was examined (Fig. 1). The left or right position of the correct stimulus on consecutive trials was determined by a Gellerman (*20*) series. Correct responses were reinforced with access to corn. Each subject was tested for 40 trials/day, 5 days/week. Learning criterion was three consecutive days for 70% or more correct responses. As each lamb met criterion on a problem, testing on the subsequent problem was begun.

The mean number of days to reach criterion for lambs within groups is given in Table 1. An analysis of variance was calculated for these data. The overall treatment effect was significant (F 10.16, df = 2, 15). The effect of problems (F 18.41, df = 5, 12)

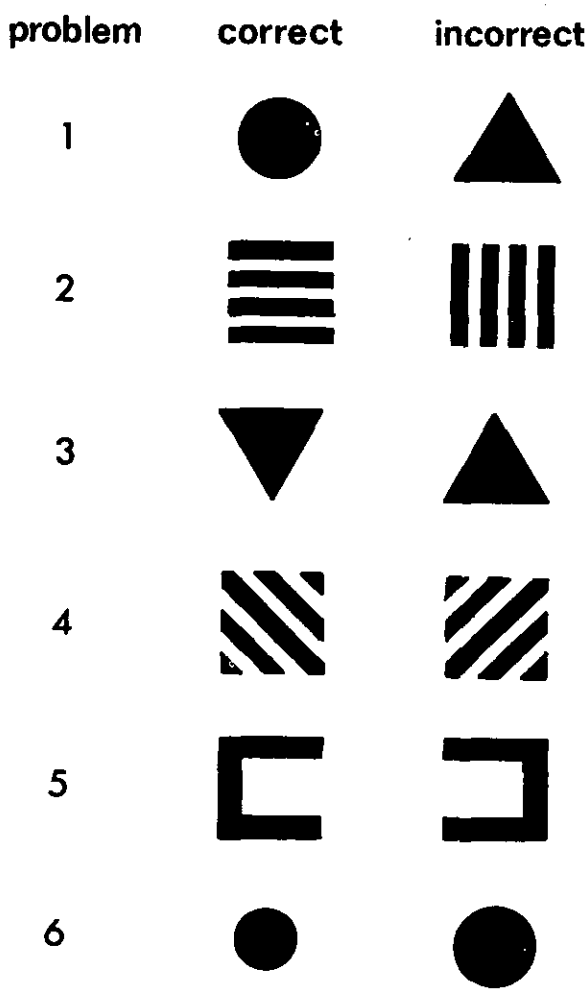

FIGURE 1. Visual discrimination problems. Two geometric form stimuli of equal area, except for problem 6, comprised each discrimination problem. The pair of stimuli for each problem were simultaneously presented as light images on dark screens. One stimulus of each pair was designated as correct. All lambs were tested in the sequence of problem number 1 through 6 consecutively.

and the treatment × problem interaction (F 3.16, df = 7, 10) was significant. An analysis of variance was calculated on days to criterion for individual problems. A significant treatment difference (F 5.48, df = 2, 15) was observed only for problem 6 (two circles of unequal size). Student's t test showed a significant difference between the control and higher lead groups (t 2.89, df = 8) and between the lower-lead and higher-lead groups (t 3.03, df = 12). Four

Table 1. Days to criterion for lambs within groups for various prenatal lead exposure groups.

Discrimination problem	Learning time, days*		
	Control	Low lead	High lead
1	6.3±0.8*	6.4±2.3	8.3± 1.8
2	4.3±1.1	4.4±0.9	4.0± 0.6
3	3.3±0.4	5.5±3.0	6.3± 5.0
4	4.8±1.3	4.4±1.4	5.3± 0.7
5	7.3±2.2	9.8±4.3	16.7± 8.5
6	12.5±5.0	13.0±9.4	29.5±10.9

*The numbers indicate the number of days (mean ± standard deviation) required by lambs within the three prenatal lead-exposure groups to learn six visual discrimination problems.

of the higher-lead lambs did not learn problem 6 in the 35-day maximum testing period. The most time required by remaining animals to learn problem 6 was 20 days.

Problem 6 was considerably more difficult for all the groups. Even though all the subjects had considerable prior experience with the discrimination task, they required more days to master this problem than any of the five previous problems. Problem 6 differed in that it involved a size discrimination rather than a form discrimination.

It is concluded that subclinical prenatal lead exposure did slow learning of a visual discrimination task in lambs when they were 10–15 months old. The results of our experiment are consistent with clinical reports of the neurologic effects of lead poisoning in children. Perlstein and Attala (*21*) reported that minimal brain damage often involved learning blocks, usually of a visual-perceptual type. Byers and Lord (*15*) found the ability to deal with shape, direction, space, and projected imagery was impaired in children recovering from lead poisoning. Thurston et al. (*16*) and Bradley and Baumgartner (*17*) reported prominent visual-motor deficits in children several years after acute lead poisoning. Jenkins and Mellins (*22*) reported that lead-poisoned children had the greatest difficulty with tasks calling for the naming of objects, visual memory, and simple conceptualizing.

These findings support recent Environmental Protection Agency (EPA) guidelines (*23*) that for pregnant women the upper ac-

ceptable blood lead level should be no more than 30 μg/100 ml. However, these results have demonstrated visual learning deficits in lambs with blood lead levels below the 40 μg/100 ml recommended by EPA (*23*) as probably safe for children.

The results of our behavioral toxicologic studies have demonstrated significant neurologic changes in sheep exposed to lead. Adult sheep were shown to have a marked day-to-day variability in performance on an auditory signal detection task. This finding parallels reports of an increased incidence of accidents to lead-exposed workers in the ship scrapping industry (*24*). Slowed learning on a visual discrimination task was demonstrated in lambs at 10–15 months of age subsequent to prenatal exposure to maternal blood lead levels of 34 μg/100 ml during gestation. This finding is consistant with the results of psychometric evaluation of children with lead poisoning.

REFERENCES

1. Wiener, G. Varying psychological sequelae of lead ingestion in children. Publ. Health Repts. 85: 19 (1970).
2. Lin-Fu, J. S. Undue absorption of lead among children—a new look at an old problem. N. Engl. J. Med. 286: 702 (1972).
3. Fine, P. R., et al. Pediatric blood lead levels, a study in 14 Illinois cities of intermediate population. JAMA 221: 1475 (1972).
4. Guinee, V. F. Lead poisoning. Am. J. Med. 52: 283. (1972).
5. Medical aspects of childhood lead poisoning. HSMHA Health Repts. 86. 140. (1971).
6. Weiss, B., and Laties, V. G. Behavioral pharmacology and toxicology. Ann. Rev. Pharmacol 9: 297 (1969).
7. Brown, S., Dragann, N., and Vogel, W. H. Effects of lead acetate on learning and memory in the rat. Arch. Environ. Health 22: 370 (1971).
8. Brown, D. R. Long-term effects of lead on learning and organ development in the growing rat. Paper presented to Society of Toxicology, 12th Annual Meeting, New York, N.Y., March 18–22 (1973).
9. Bullock, J. D., et al. Effect of tetraethyllead on learning and memory in the rat. Arch. Environ. Health 13: 21 (1966).
10. Goode, J. W., Johnson, S., and Calandra, J. C. Evaluation of chronic oral administration of lead acetate to Rhesus monkeys. Paper presented to Society of Toxicology, 12th Annual Meeting, New York, N.Y., March 18–22 (1973).
11. Van Gelder, G. A., et al. Behavioral toxicologic assessment of the neurologic effect of lead in sheep. Clin. Toxicol. 6: 405 (1973).
12. Rabinovitch, M. S., and Rosvold, H. A closed-field intelligence test for rats. Can. J. Psychol. 5: 122 (1951).
13. Hebb, D. O., and Williams, K. A method of rating animal intelligence. J. Genet. Psychol. 34: 59 (1946).
14. Carson, T. L., et al. Effects of low level lead ingestion in sheep. Clin. Toxicol 6: 389 (1973).
15. Byers, R. K., and Lord, E. E. Late effects of lead poisoning on mental development. Am. J. Dis. Child. 66: 471 (1943).
16. Thurston, D. L., Middlekamp, J., and Mason, E. The late effects of lead poisoning. J. Pediatr. 47: 413. (1955).
17. Bradley, J. E., and Baumgartner, R. J. Subsequent mental development of children with lead encephalopathy as related to type of treatment. J. Pediatr. 53: 311 (1958).
18. Carson, T. L., et al. Slowed learning in lambs prenatally exposed to lead. Arch. Environ. Health, in press.
19. Schnorr, J. K. Effects of dieldrin on learning and retention of a visual discrimination task in sheep. Ph.D. thesis, Iowa State University, Ames, Iowa, 1972; Microfilm #73-3928. Xerox University Microfilms, P.O. Box 1764, Ann Arbor, Michigan.
20. Gellerman, I. W. Chance order of alternating stimuli in visual discrimination experiments. J. Genet. Psychol. 42: 206 (1933).
21. Perlstein, M. A., and Attala, R. Neurologic sequelae of plumbism in children. Clin. Pediat. 5: 292 (1966).
22. Mellins, R. B., and Jenkins, C. D. Epidemiological and psychological study of lead poisoning in children. JAMA 158: 15 (1955).
23. Environmental Protection Agency, Health Effects Branch, Processes and Effects Division, Office of Research and Monitoring. EPA's position on health effects of airborne lead. Washington, D.C., 1972.
24. Rieke, F. E. Lead intoxication in shipbuilding and shipscrapping 1941–1968. Arch. Environ. Health 19: 521 (1969).